ISOLATION, STRUCTURE ELUCIDATION, AND SYNTHESIS OF THE MAJOR ANAEROBIC BACTERIAL METABOLITE OF THE DIETARY CARCINOGEN 2-AMINO-3,4-DIMETHYL-3H-IMIDAZO[4,5-f]QUINOLINE (MeIQ).

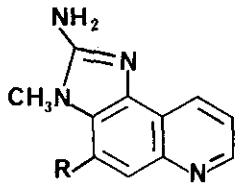
Mohammad Bashir and David G. I. Kingston*
Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212, U.S.A.
Robert J. Carman, Roger L. Van Tassell, and Tracy D. Wilkins*
Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0305, U.S.A.

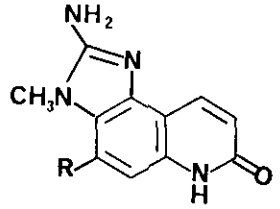
Abstract - Incubation of the heterocyclic cooked food mutagen 2-amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline (MeIQ, 3) with mixed human fecal microflora under anaerobic conditions yielded 2-amino-3,6-dihydro-3,4-dimethyl-7H-imidazo[4,5-f]quinolin-7-one (HOMeIQ, 4) as the major detectable metabolite. HOMeIQ (4) was synthesized in six steps from 6-bromo-7-methylquinoline (5).

The importance of dietary factors in the epidemiology of colon cancer has become increasingly recognised over the last several years.[1] The heterocyclic pyrolysis products formed on cooking meat and other proteinaceous foods have been recognised as major contributors to the total mutagenic and/or carcinogenic burden,[2] and have been shown to be responsible for the rise in fecal mutagenicity upon ingestion of fried beef.[3] Because of the importance of these heterocyclic carcinogens, it becomes necessary to understand their metabolism in the human gastrointestinal tract, so that the full extent of the risk of these compounds to human health can be understood and, perhaps, alleviated.

In earlier studies we reported on the anaerobic metabolism of the potent mutagen and carcinogen 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ, 1) to 2-amino-3,6-dihydro-3-methyl-7H-imidazo[4,5-f]quinolin-7-one (HOIQ, 2),[4] on the syntheses of HOIQ (2),[5] on the microbiological conversion of IQ (1) to HOIQ (2) and on the mutagenicity of HOIQ (2),[6] and on the in vivo conversion of IQ (1) to HOIQ (2).[7] Particularly significant is the finding that HOIQ (2) is a potent direct-acting mutagen, while IQ (1) itself requires metabolic activation.[6]

The pyrolysis product 2-amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline (MeIQ, 3) is one of the most





1 R=H 3 R=CH_3 2 R=H 4 R=CH_3

potent bacterial mutagens known.[2e] First reported by Sugimura and his co-workers,[8] it is a potent carcinogen in mice[9] and rats.[10] It requires metabolic activation for it to show significant activity.[11] We now report that MeIQ (**3**) undergoes microbial transformation by human fecal bacteria to 3,6-dihydro-3,4-dimethyl-7H-imidazo[4,5-f]quinolin-7-one (HOMeIQ, **4**), and we also report a synthesis of HOMeIQ (**4**).

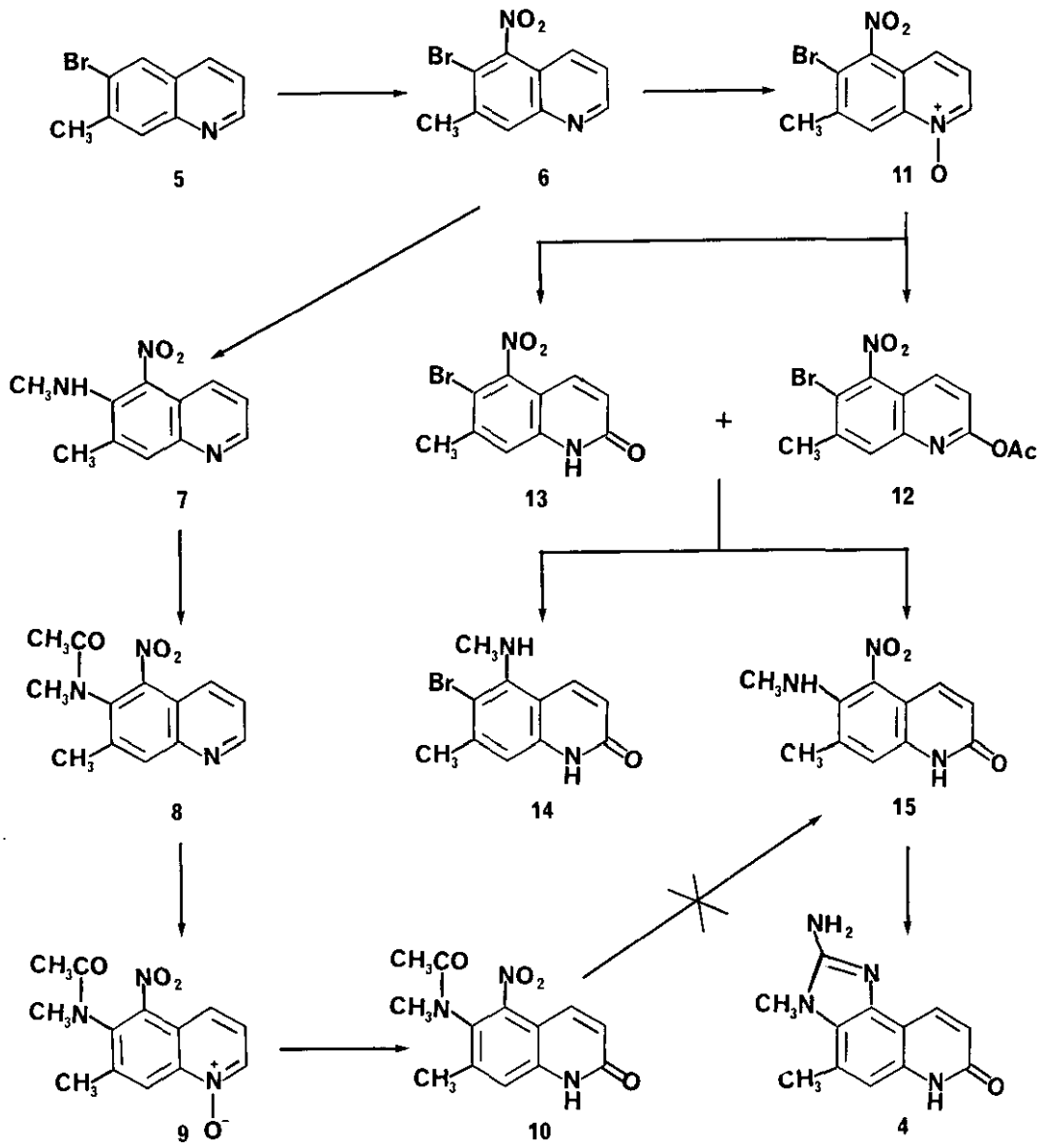
RESULTS AND DISCUSSION

Incubation of [^{14}C]MeIQ (**3**) with diluted human feces or pure cultures of Eubacterium moniliforme as previously described,[4,6] followed by extraction of aromatic metabolites with blue cotton,[4,12] yielded one major metabolite as determined by radioautography. Incubation of [^{14}C]MeIQ (**3**) for up to 8 days with autoclaved feces or with BHI broth alone yielded unchanged starting material, indicating that the new compound observed was a true bacterial metabolite and not a chemical decomposition product.

Sufficient quantities of the metabolite for chemical work were obtained by incubating unlabeled MeIQ (**3**) under identical conditions to those used for the labeled compound. The major metabolite was isolated by extraction with blue cotton followed by a final C18-hplc purification; the isolated metabolite was chromatographically identical with the ^{14}C-metabolite on tlc. The ^{1}H nmr spectrum of the metabolite showed the presence of one pair of ortho protons (pair of doublets at 8.27, 6.43 ppm, J = 9.6 Hz) and an aromatic singlet at 6.66 ppm; singlets for two isolated methyl groups were observed at 2.64 and 3.72 ppm. The mass spectrum showed a molecular ion at m/z 228, corresponding to the composition $C_{12}H_{12}N_4O$, or MeIQ (**3**) + O.

Based on this evidence, and by analogy with the structure of the metabolic product formed from IQ (**1**),[4,5] the metabolite was assigned the structure 2-amino-3,6-dihydro-3,4-dimethyl-7H-imidazo[4,5-f]quinolin-7-one, or HOMeIQ (**4**).

Confirmation of structure **4** for the metabolite was obtained by synthesis. The first approach was modeled on our successful synthesis of HOIQ (**2**).[5] Nitration of 6-bromo-7-methylquinoline (**5**) yielded the 5-nitro derivative **6**, which was converted to the amine **7** as previously described.[13] Protection of the amine as its acetyl derivative gave the amide **8**, and oxidation with m-chloro-


Br
CH3
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5
NO2
6
11
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CH3NH
7
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OAc
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CH3CO
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14
15
9
10
NH2
4

perbenzoic acid (MCPBA) gave the N-oxide **9** in good yield. Rearrangement of **9** to the quinolone **10** proceeded smoothly, but hydrolysis of **10** to the desired amine **15** was not successful. Hydrolysis under mild acid or base conditions yielded unchanged starting material while hydrolysis under more vigorous conditions gave products which had lost the nitro group.

This hydrolytic step had proved troublesome in our synthesis of HOIQ (**2**),[5] but careful control of conditions had enabled us to make it work. In the present case, the additional ortho substituent to the acetylamino group presumably prevents the latter from achieving coplanarity with the ring, and thus prevents conjugation of the amide nitrogen lone pair with the nitro group, thus making nucleophilic displacement of this nitrogen more difficult.

We thus turned to an alternate route which successfully yielded HOMeIQ (**4**), albeit in low yield. Oxidation of the nitroquinoline **6** with MCPBA yielded the N-oxide **11**, which rearranged to a mixture of the quinolin-7-one **13** and the acetoxyquinoline **12** on heating with acetic anhydride. Treatment either of **12** alone or of the mixture of **12** and **13** with methylamine yielded a mixture of the displacement products **14** and **15**, with the desired product **15** in slightly higher yield; we were unfortunately unable to find conditions under which selective displacement of bromine occurred. Final conversion of the nitroamine **15** to HOMeIQ (4) was achieved by reduction with Raney nickel and hydrogen, followed by treatment of the resulting diamine with bromocyanogen. The product **4** was identical in all respects (^{1}H nmr, ms, tlc, hplc) with the product obtained by metabolism of MeIQ (**2**). The relative mobilities of MeIQ (**3**) and HOMeIQ (**4**) on tlc were 0.72 and 0.42 respectively; on hplc their k´ values were 6.3 and 12.6 respectively.

EXPERIMENTAL

General

General experimental procedures were as previously described.[4,5] ^{1}H-Nmr chemical shifts are reported in parts per million from internal tetramethylsilane.

Synthesis of [2-^{14}C]MeIQ (3)

[2-^{14}C]MeIQ (**3**) was prepared by the method of Adolfsson.[13]

Incubation of MeIQ (3) with feces and pure cultures

MeIQ (**3**) was incubated with fresh whole feces and pure cultures of Eubacterium monoliforme VPI 13480 as previously described for in vitro production of HOIQ (**2**) from IQ (**1**).[4,6] Ten grams of feces or 1 ml of an 18 h culture of E. moniliforme were resuspended in 10 ml of brain heart infusion broth supplemented with 10-50 µg/ml MeIQ (**3**). The suspensions were incubated anaerobically at 37°C for 2-5 days.

Extraction and isolation of the metabolite

The metabolite was extracted from the diluted feces and pure cultures with blue cotton as previously described.[4,6] The metabolite was purified from the blue cotton extracts by reverse phase hplc and characterized by tlc as previously described.[6] The reverse phase system consisted of a Waters Radial Compression Module and a Nova-Pak C_{18} cartridge with methanol/water/ammonium hydroxide (40:60:2). Peaks were recorded and integrated by an HP3390A integrating recorder. The tlc system consisted of silica gel plates with chloroform/methanol/ammonium hydroxide (85:15:1).

Metabolite structure

The metabolite had the following spectroscopic properties: ^{1}H nmr (CD_3OD) 8.27 (1H, d, J = 9.6 Hz), 6.66 (1H, s), 6.43 (1H, d, J = 9.6 Hz), 3.72 (3H, s), 2.64 (3H, s); eims m/z (relative intensity) 228($M^{+\cdot}$,4), 213(1.5), 199(1), 185(3), 177(3), 158(2); uv (CH_3OH) λ_{max} 362 nm, 333, 273, 230.

7-Methyl-6-bromo-5-nitroquinoline (6).

6-Bromo-7-methylquinoline[15] (10 g, 45.24 mmol) was added to an ice cooled mixture of concentrated H_2SO_4 (20 ml) and 90% HNO_3 (2.45 ml). After being stirred for 2 h below 20°C, the reaction mixture was added to ice and made basic with NH_4OH. The basic aqueous solution was extracted with $CHCl_3$, and the organic layers were combined, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (CH_2Cl_2) yielded **6** (7.3 g, 68%) mp 130-133°C(MeOH). ^{1}H Nmr ($CDCl_3$) 2.69 (3H, s), 7.52 (1H, dd, J_1 = 8.9 Hz, J_2 = 4.3 Hz), 8.13 (1H, s), 8.99 (1H, d, J = 4.3 Hz); eims m/z (relative intensity) 268, 266 ($M^{+\cdot}$, 46, 50), 238, 236 (30, 32), 222, 220 (20,22), 210, 208 (16, 18), 187 (13), 141(100); uv λ_{max} 325 nm (ε 12,000), 310 (10,600), 270 (10,000), 230 (111,000); ir ($CHCl_3$) 1580, 1540, 1460, 1360, 1310 cm^{-1}. Anal. Calcd for $C_{10}H_7O_2Br$: C, 44.94; H, 2.62; N, 10.48. Found: C, 44.80; H, 2.61; N, 10.06.

7-Methyl-6-methylamino-5-nitroquinoline (7).

Methylamine (10g 40% aqueous, 322 mmol) was added dropwise over 1 h to a refluxing solution of **6** (5g, 18.79 mmol) in ethanol (60 ml). After refluxing for another 4 h tlc showed the absence of starting material. The solution was then poured into ice water and extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4) and evaporated. Crystallization of the residue from MeOH yielded **7** (3g, 75%), mp 159-160°C (lit.[13] 161-162°C). ^{1}H Nmr ($CDCl_3$) 2.45 (3H, s), 3.00 (3H, d, J = 5.3 Hz), 5.25 (1H, br s), 7.40 (1H, dd, J_1 = 8.7 Hz, J_2 = 4.2 Hz), 7.88 (1H, s), 8.18 (1H, d, J = 8.7 Hz), 8.69 (1H, d, J = 4.2 Hz); eims m/z (relative intensity) 217 ($M^{+\cdot}$, 100), 200 (35), 183 (20), 170 (45), 156 (40), 143 (65), 129 (20), 115 (45); uv λ_{max} 420 nm (ε 6500),

350 (21,700), 290 (17,400), 250 (106,300); ir ($CHCl_3$) 3400, 2920, 1610, 1470, 1335, 1250, 1150 cm^{-1}. Hrms for $C_{11}H_{11}N_3O_2$: M 217.0851. Found: M 217.0854.

7-Methyl-6-(N-acetyl-N-methyl)-5-nitroquinoline (8).

Compound **7** (2.5 g, 11.5 mmol) was dissolved in acetic anhydride (30 ml) and pyridine (5 ml) and refluxed for 6 h in an N_2 atmosphere. Solvent was removed in vacuo and the residue was dissolved in $CHCl_3$ and washed with water. The organic layer was dried (Na_2SO_4) and evaporated. Purification by flash chromatography ($CHCl_3$, then $CHCl_3$; MeOH, 90:10) afforded **8** (2.5 g, 84%, mp 122-125°C (ether). ^1H Nmr ($CDCl_3$) 1.81 (3H, s), 2.47 (3H, s), 3.19 (3H, s), 7.54 (1H, dd, J_1 = 8.6 Hz, J_2 = 4.2 Hz), 8.02 (1H, dd, J_1 = 8.6 Hz, J_2 = 4.2 Hz); eims m/z (relative intensity) 260 ($M+1^{+\cdot}$, 100), 230 (45), 212 (50), 184 (6); uv λ_{max} 320 nm (ε 7,300), 312 (6,200), 225 (72,500); ir ($CHCl_3$) 1690, 1620, 1550, 1500, 1400, 1340 cm^{-1}. Anal. Calcd for $C_{13}H_{13}N_3O_4$: C, 60.23; H, 5.01; N, 16.21. Found: C, 60.09; H, 4.89; N, 16.18.

7-Methyl-6-(N-acetyl-N-methyl)-5-nitroquinoline-N-oxide (9).

A solution of m-chloroperbenzoic acid (2.64 g, 15.34 mmol) in 50 ml of $CHCl_3$ was added dropwise over 2 h to a stirred solution of **8** (2.2 g, 8.49 mmol) in 10 ml of $CHCl_3$ below 0°C in an N_2 atmosphere. The reaction mixture was stirred for an additional 4 h at the same temperature and then kept overnight at 0°C. The solution was then treated with a saturated solution of $NaHCO_3$ and finally with water. The organic layer was dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography ($CHCl_3$, then $CHCl_3$:MeOH, 98:2) gave **9** (2g, 87%) as a yellow solid which was recrystallized from EtOH, mp 160-163°C. ^1H Nmr ($CDCl_3$) 1.84 (3H, s), 2.53 (3H, s), 3.21 (3H, s), 7.45 (1H, dd, J_1 = 8.6 Hz, J_2 = 5.9 Hz), 7.54 (1H, d, J = 8.6 Hz), 7.45 (1H, dd, J_1 = 8.6 Hz, J_2 = 5.9 Hz), 7.54 (1H, d, J = 8.6 Hz), 8.59 (1H, d, J = 5.9 Hz), 8.91 (1H, s); eims m/z (relative intensity) 275 ($M^{+\cdot}$, 3), 229 (100), 212 (40), 186 (12), 142 (20); uv λ_{max} 350 nm (ε 8,200), 252 (23,400), 232 (42,600), 225 (41,200); ir ($CHCl_3$) 3020, 1690, 1550, 1440, 1390, 1370, 1270 cm^{-1}. Anal. Calcd. for $C_{13}H_{13}N_3O_4 \cdot 1/2\ H_2O$: C, 54.92; H, 4.57; N, 14.78. Found: C, 55.12; H, 4.67; N, 14.67.

7-Methyl-6-(N-acetyl-N-methyl)-5-nitroquinolin-2-one (10).

A solution of **9** (1.17 g, 6.18 mmol) in 50 ml of acetic anhydride was refluxed for 23 h in an N_2 atmosphere. Solvent was then removed in vacuo and the residue was basified with NH_4OH and extracted with $CHCl_3$. The organic phases were combined, dried (Na_2SO_4) and evaporated. Purification by flash chromatography ($CHCl_3$, then $CHCl_3$: MeOH, 95:5) yielded **10** (1g, 58%), which was crystallized from EtOH, mp 238-240°C. ^1H Nmr ($CDCl_3$) 1.81 (3H, s), 2.39 (3H, s), 3.14 (3H,

s), 6.84 (1H, d, J = 9 Hz), 7.52 (1H, s), 7.66 (1H, d, J = 9 Hz), 11.80 (1H, br s); eims m/z (relative intensity) 275 ($M^{+\cdot}$, 1), 229 (100), 214 (10), 199 (10), 186 (20), 167 (10), 158 (20), 142 (25), 130 (30); uv λ_{max} 340 nm (ε 3,700), 280 (2,700), 235 (20,600); ir ($CHCl_3$) 2900, 1660, 1520, 1440, 1340, 1270 cm^{-1}. Anal. Calcd for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.72; N, 15.27. Found: C, 56.41; H, 4.75; N, 15.12.

7-Methyl-6-bromo-5-nitroquinoline-N-oxide (11).

Compound **6** (1g, 3.75 mmol) was dissolved in 10 ml of $CHCl_3$ and cooled to below 0°C. A solution of m-chloroperbenzoic acid (1.099 g, 6.38 mm) in 30 ml of $CHCl_3$ was added dropwise over 2 h at the same temperature. Stirring was continued for 4 h, and the reaction mixture was kept overnight at 0°C. The resulting solution was washed with saturated $NaHCO_3$ and then with water. The chloroform layer was dried ($MgSO_4$) and evaporated in vacuo. Purification by flash chromatography (CH_2Cl_2 then CH_2Cl_2:MeOH, 98:2) afforded **11** (0.945 g, 90%), mp 178-182°C (EtOH). ^1H Nmr ($CDCl_3$) 2.72 (3H, s), 7.36-7.47 (2H, m), 8.55 (1H, d, J = 5.8 Hz), 8.79 (1H, s); eims m/z (relative intensity) 284, 282 ($M^{+\cdot}$, 100, 100), 254, 252 (10, 12), 238, 236 (30, 32), 182 (10), 157 (15), 140 (35), 129 (40), 102 (38); uv λ_{max} 340 nm (ε 5,600), 255 (17,600), 235 (27,500); ir ($CHCl_3$) 1560, 1400 1340, 1250 cm^{-1}. Anal. Calcd for $C_{10}H_7O_3N_2Br$: C, 42.40; H, 2.47; N, 9.89. Found: C, 42.46; H, 2.49; N, 9.57.

7-Methyl-6-bromo-5-nitroquinolin-2-one (12).

A solution of **11** (0.6g, 2.12 mmol) in 25 ml of acetic anhydride was refluxed for 15 h in an N_2 atmosphere. Solvent was removed in vacuo and the residue was basified with NH_4OH and extracted with $CHCl_3$. The chloroform layers were combined, dried (Na_2SO_4) and evaporated, and the product was purified by flash chromatography ($CHCl_3$). Early fractions afforded **12** (150 mg, 21%), mp 130-133°C (EtOH). ^1H Nmr ($CDCl_3$) 2.42 (3H, s), 2.68 (3H, s), 7.32 (1H, d, J = 8.9 Hz), 8.01 (1H, s), 8.03 (1H, d, J = 8.9 Hz); eims m/z (relative intensity) 326, 324 ($M^{+\cdot}$, 8,10), 284, 282 (98, 100), 238, 236 (30, 30), 226, 224 (20, 20), 210, 208 (15, 16), 173 (10), 157 (20), 129 (35), 102 (30); uv λ_{max} 325 nm (ε 8,100), 310 (6,500), 235 (82,600); ir ($CHCl_3$) 1760, 1580, 1560, 1350, 1160 cm^{-1}.

Later fractions of the column afforded **13** (200 mg, 33%) which was crystallized from MeOH as light brown needles, mp > 300°C. ^1H Nmr (DMSO) 2.48 (3H, s), 6.63 (1H, d, J = 9.8 Hz), 7.42 (1H, s), 7.57 (1H, d, J = 9.8 Hz) 12.27 (1H, s); eims m/z (relative intensity) 284, 282 ($M^{+\cdot}$, 100, 100), 238, 236 (40, 45), 226, 224 (15, 18), 210, 208 (20, 25), 173 (10), 157 (30), 129 (50), 102 (40); uv λ_{max} 340 nm (ε 12,200), 280 (6,600), 240 (6,900); ir (KBr) 1655, 1510, 1350, 1180 cm^{-1}. Anal. Calcd for $C_{10}H_7O_3N_2Br$: C, 42.40; H, 2.47; N, 9.89. Found: C, 42.43; H, 2.50; N, 9.49.

7-Methyl-6-methylamino-5-nitro-2(1H)-quinoline (**15**).

Compound **12** (200 mg, 0.709 mmol) in 6 ml of EtOH and 33% methylamine in EtOH (2 ml) were heated in a sealed tube for 6 h at 120°C. After this time solvent was removed *in vacuo*. The products were purified by flash chromatography (EtOAc, then EtOAc:MeOH, 90:10). Early fractions afforded **14** (40 mg, 21%), mp 194-196°C (EtOH). ^{1}H Nmr ($CDCl_3$) 2.47 (3H, s), 2.97 (3H, s), 6.60 (1H, d, J = 9.8 Hz), 6.89 (1H, s), 8.10 (1H, d, J = 9.8 Hz), 11.69 (1H, s); eims m/z (relative intensity) 268, 266 ($M^{+\cdot}$, 100, 100), 238, 236 (22, 20), 224, 222 (20, 18), 187 (50), 172 (30), 158 (55), 130 (62), 117(40), 102(55); uv λ_{max} 320 nm (ε 5,300), 265 (9,300), 245 (14,600), 220 (18,600); ir ($CHCl_3$) 3300, 1650, 1440, 1360 cm^{-1}. Later fractions yielded **15** (40 mg, 24%) as an orange solid which was recrystallized from MeOH, mp 232-235°C. ^{1}H Nmr ($CDCl_3$) 2.44 (3H, s), 2.88 (3H, s), 4.56 (1H, br s), 6.77 (1H, d, J = 9.8 Hz), 7.36 (1H, s), 7.87 (1H, d, J = 9.8 Hz), 12.15 (1H, br s); eims m/z (relative intensity) 233 ($M^{+\cdot}$, 100), 216 (25), 199 (20), 187 (25), 172 (25), 158 (45), 142 (30), 130 (40), 117 (10), 103 (30), 77 (36); uv λ_{max} 380 nm (ε 9,300), 240 (72,200); ir ($CHCl_3$) 2900, 1660, 1620, 1580, 1515, 1440, 1345 cm^{-1}. Anal. Calcd for $C_{11}H_{11}N_3O_3$: M 233.0800. Found: M 233.0794.

HOMeIQ (**4**).

A stirred mixture of **15** (20 mg, 0.085 mmol), Raney nickel (1/4 spatula) and ethanol (5 ml) was hydrogenated at room temperature for 15 min. The catalyst was then filtered off and the filtrate was treated with BrCN (20 mg, 0.188 mmol) and stirred overnight at room temperature, after which the solution was basified with NH_4OH and evaporated. Purification by preparative tlc ($CHCl_3$: MeOH : NH_4OH, 80 : 20 : 1) gave **4** (12.5 mg, 64%), mp > 340°C (EtOH). ^{1}H Nmr (CD_3OD) 2.65 (3H, s), 3.74 (3H, s) 6.45, (1H, d, J = 4.3 Hz), 6.69 (1H, s), 8.25 (1H, d, J = 9.3 Hz); eims m/z (relative intensity) 228 ($M^{+\cdot}$, 40), 213 (22), 199 (15), 185 (20), 177 (12), 158 (10), 114 (12), 72 (35), 54 (100); uv λ_{max} 360 nm (ε 5,100), 330 (17,100), 320 (18,500), 270 (23,700), 227 (50,200); ir (KBr) 3300, 3120, 1620, 1425, 1360, 1180 cm^{-1}. The isolated product was identical (^{1}H nmr, ms, tlc, hplc) with metabolite isolated from feces.

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